Pharmacokinetics and immunogenicity of TNF-inhibitors, towards optimised treatment of rheumatoid arthritis

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Patients non-responding to etanercept obtain lower etanercept concentrations compared to responding patients

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ABSTRACT

Objective To investigate the relationship between serum etanercept levels and clinical response.

Methods In 292 etanercept-treated patients with rheumatoid arthritis clinical and pharmacological data were determined at baseline and after 1, 4 and 6 months of etanercept treatment. Differences in etanercept levels between good, moderate and European League Against Rheumatism (EULAR) non-responders were assessed after 6 months of therapy.

Results After 6 months of therapy etanercept levels were significantly higher in good responders (median (IQR)) 3.78 (2.53-5.17)) compared with both moderate 3.10 (2.12-4.47) and EULAR non-responders 2.80 (1.27-3.93) (all $p<0.05$). There was a significant association between clinical response and serum etanercept levels (regression coefficient=0.54; 95%CI: 0.21 to 0.86, p=0.001). When patients were categorized into quartiles according to the height of etanercept levels, the lowest quartile (etanercept level <2.1mg/L) comprised 40% of all non-responders. The highest quartile (etanercept level >4.7mg/L) comprised 35% of all EULAR good responders. Anti-etanercept antibodies were detected in none of the sera.

Conclusion The authors demonstrated that lower etanercept levels were associated with non-response. Therapeutic drug monitoring and the possibility of the adjusted dosing regimens in the selected groups of patients should be investigated further as a possible tool to optimize treatment with etanercept.

INTRODUCTION

Although the efficacy of etanercept 50 mg per week has been demonstrated, a low number of patients achieve clinical remission.[1] Furthermore, administration of higher etanercept doses did not lead to additional efficacy in patients with suboptimal response to the standard dose of 50 mg per week.[2] Until now, decision making in the case of failure to anti-tumour necrosis factor (TNF) treatment is based on clinical outcome alone, without taking into account the circulating drug levels.[3] However pharmacological and disease-related aspects may both influence drug efficacy.[4] For example, a low synovial expression of TNFα has been associated with lack of response to anti-TNF treatment.[5]

Previously, an association between low circulating drug levels and lack of clinical response was demonstrated for infliximab- and adalimumab-treated patients.[6 7] In contrast, no association was found between etanercept drug levels and clinical response.[2 8] Furthermore, antibodies against etanercept, all non-neutralizing, were measured in less than 2% of the patients.[8-10] In rheumatoid arthritis (RA) patients, a lower response to etanercept was associated with high level of disability, the presence of IgM rheumatoid factor and etanercept monotherapy.[11 12]

Although a personalized treatment strategy has been proposed for patients treated with TNF-inhibitors,[3 6] the clinical consequence of monitoring circulating etanercept levels is not yet clear. Therefore, we aimed to investigate the association between circulating etanercept levels and clinical response in a large cohort of etanercept-treated RA patients.

PATIENTS AND METHODS

Study population

The study population consisted of patients with RA, all treated with etanercept, included in an observational cohort. Inclusion criteria for this cohort were RA according to the American College of Rheumatology 1987 criteria,[13] age 18 years or older, failure on at least two disease-modifying antirheumatic drugs (DMARDs) including methotrexate[14] and active disease as measured by the disease activity score in 28 joints (DAS28) of more than 3.2. Patients were treated with either concomitant medication, including methotrexate and prednisone, or etanercept monotherapy. All patients used etanercept 50 mg subcutaneously every week or 25 mg twice a week. None of the patients received a dose increase of

etanercept during the 6 months of the observation period. The study was approved by the local medical ethics committee and all patients gave written informed consent.

Clinical response to etanercept

Disease activity was assessed at baseline and after 1, 4 and 6 months of therapy by the DAS28. Clinical response was assessed using the European League Against Rheumatism (EULAR) response criteria.[15]

Measurement of serum trough levels of etanercept

Serum etanercept levels were measured using ELISA. The sensitivity of detection is 1 ng/ml (=0.001mg/l). All samples were collected prior to the next etanercept injection at baseline, 1, 4 and 6 months of etanercept treatment. As described previously this assay is based on the ability of etanercept to bind TNF.[8] Briefly, a mouse monoclonal anti-TNF antibody was preincubated overnight onto microtitre plates. Thereafter recombinant TNF in high-performance ELISA (HPE) buffer was added. After one hour plates were washed with phosphate buffered saline 0.02% Tween. Subsequently patient serum sample in different dilutions was added and incubated for 1 hour at 37°C. After washing the plates with phosphate buffered saline 0.02% Tween plates were incubated with biotinylated polyclonal rabbit antibodies against etanercept in 100 µl HPE buffer for 1 hour at 37°C. After plates were washed poly(horseradish peroxidise)-conjugated streptavidin was added for 30 min at 30°C, followed by incubation with tetramethylbenzidine (TMB). Afterwards reaction was stopped and absorption at 450 nm was assessed. Test results were reading out of a titration curve of etanercept, which was present in each plate.

Measurement of antibodies against etanercept

Antibodies against etanercept were measured by 2-site radio immune assay (RIA), bridging ELISA and IgG4-ABT.

2-site radio immune assay (RIA) Etanercept coupled to sepharose was used to bind anti-etanercept antibodies. One µl of patient serum was overnight incubated with sepharose. Subsequently non-bound serum was washed off and 125I- radiolabeled etanercept was added. After one night incubation non-bound radiolabel was removed by washing. Serum from an etanercept-vaccinated rabbit served as positive control. Different dilutions of rabbit anti-etanercept serum were used to read the results.

Bridging ELISA Microtiter plates were incubated overnight with 0.06 µg/ml etanercept in PBS and subsequently washed five times with PBS/0.02% Tween (PT) and incubated with patient serum which was serial diluted in high-performance ELISA buffer (HPE, Business Unit Reagents, Sanquin) for one hour. The highest concentration was 10%. All incubations were performed at room temperature in an assay volume of 100 µl. Next, the plates were washed with PT and incubated with 3 ug/ml biotinylated etanercept in HPE for one hour. After washing the ELISA was developed using 100 µg/ml tetramethylbenzidine in 0.11M sodium acetate (pH 5.5) containing 0.003% (v/v) H2O2. The reaction was stopped with 2M H2SO4. Absorption at 450 nm was measured with a multiscan (Multiskan; Titertek, Elfab Oy, Finland). Results were related to a titration curve of etanercept-specific rabbit serum containing anti-etanercept antibodies and expressed in arbitrary units (AU).

IgG4-Antigen binding test Antibodies to etanercept of the IgG4 subclass were measured essentially as described by previously.[8] In brief, anti-human IgG4 monoclonal antibody coupled to sepharose was incubated with patient serum. Antibodies were detected by incubation with 125I labeled etanercept. Results were related to a titration curve of etanercept-specific rabbit serum containing anti-etanercept antibodies and expressed in arbitrary units (AU).

Statistical analysis

Data are shown as mean (SD), median (IQR) or percentages. Differences between good, moderate and non-responders were evaluated by using an independent t-test, $\chi 2$ or Mann-Whitney U test, if appropriate. Analyses were performed using the method of last observation carried forward for patients who discontinued the treatment with etanercept before 6 months. To investigate whether etanercept levels were influenced by confounders multiple regression analyses were performed. All baseline variables were considered as potential confounders and included in the model if the β changed 10% or more. Logistic regression analyses were performed to examine the association between height of the serum etanercept levels and EULAR response. p Values less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics and clinical response

Clinical and demographic characteristics of all patients are shown in table 1. Eighty per cent of the patients (234/292) completed 6 months of etanercept treatment. Of the 58 patients who discontinued etanercept treatment before 6 months, 35 patients stopped due to inefficacy, 15 because of adverse events and eight patients stopped for reasons such as relocation, unwillingness to participate and lost to follow-up.

After 6 months of etanercept treatment, 103 (36%) patients were good, 115 (39%) were moderate and 74 (25%) were non-responders according to the EULAR response criteria (table 1).

Changes in serum etanercept levels

At baseline, serum etanercept levels were undetectable. Median (IQR) etanercept levels in all patients were 3.17mg/L (1.90-4.33), 3.25mg/L (2.08-4.71) and 3.44mg/L (2.34-4.78) after 1, 4 and 6 months of etanercept treatment, respectively.

When stratified for EULAR response serum etanercept levels were significantly higher in good responders compared with both moderate and EULAR non-responders at all time points (all $p<0.05$). There were no statistical differences in etanercept levels between moderate and EULAR non-responders: $p=0.93$, $p=0.09$ and $p=0.05$ at 1, 4 and 6 months respectively (table 2).

A sensitivity analysis was performed for patients who completed 6 months follow-up, this did not alter the results (data not shown).

Table 1. Demographic and clinical characteristics at baseline

	All patients (n=292)	Good responders^ (n=103)	Moderate responders^ (n=115)	Non-responders^ (n=74)
Demographics				
Age	52.8 ± 12.7	50.5 ± 11.7†	54.4 ± 12.7†	53.7 ± 13.8
Female, n (%)	239 (82)	79 (77)	95 (83)	65 (88)
Body mass index	26.0 ± 5.6	25.9 ± 5.4	25.7 ± 5.2	26.7 ± 6.5
Smoking, n (%)	77 (27)	32 (32)	29 (26)	16 (22)
Glomerular filtration rate[1]	118.6 ± 39.3	121.5 ± 38.1	114.6 ± 39.8	120.6 ± 40.7
DMARD and prior biological therapy				
Previous DMARD	2.9 ± 1.2	2.7 ± 1.1*†	3.0 ± 1.4†	3.0 ± 1.3*
Methotrexate use, n (%)	223 (76)	82 (80)	87 (76)	54 (73)
Methotrexate dose, mg/week	19.7 ± 7.0	20.9 ± 6.3	19.3 ± 7.5	18.8 ± 7.2
Prednisone use, n (%)	83 (28)	26 (25)	35 (30)	22 (30)
Prednisone dose, mg/day	8.2 ± 3.9	7.3 ± 4.1	8.5 ± 3.9	8.6 ± 3.7
Other DMARD than methotrexate, n (%)	96 (33)	41 (40)	34 (30)	21 (28)
Previous biological agent, n (%)[2]	89 (31)	19 (18)*†	41 (36)†	29 (39)*
Disease status				
Disease duration (years)	8 (3-16)	8 (2-14)†	9 (3-18)†	7 (3-17)
Rheumatoid factor, n (%)	207 (72)	74 (73)	84 (74)	49 (66)
Erosive disease, n (%)	206 (72)	70 (69)	83 (74)	53 (72)
HAQ	1.3 ± 0.7	1.2 ± 0.8	1.4 ± 0.7	1.3 ± 0.7
DAS28	5.2 ± 1.3	5.0 ± 1.2†	5.7 ± 1.2†#	4.7 ± 1.5#
Erythrocyte sedimentation rate, mm/h	23 (12- 0)	17 (9-31)†	29 (14-46)†#	20 (10-40)#
C-reactive protein, mg/l	8 (3-21)	6 (3-20)	11 (4-23)#	6 (2-17)#

Mean values ± SD, median and IQR, or percentages are shown.
^last observation carried forward data were used for patients who discontinued the treatment with etanercept before 6 months
†There was a significant difference between good and moderate EULAR responders for age (p=0.017); previous DMARD (p=0.0034); DAS28 (p<0.001); previous biological agent use (p=0.005); disease duration (p=0.038) and erythrocyte sedimentation rate (p<0.001).
*There was a significant difference between EULAR good and non-responders for previous DMARD (p=0.047) and previous biological agent use (p=0.002).
#There was a significant difference between moderate and EULAR non-responders for DAS28 (p<0.001); erythrocyte sedimentation rate (p=0.039); C-reactive protein (p=0.033).
[1]Glomerular filtration rate according to Cockcroft-Gault formula
[2]Previous biological agents consisted of infliximab and adalimumab
DAS28, disease activity score in 28 joints; DMARD, disease-modifying antirheumatic drug; EULAR, European League Against Rheumatism; HAQ, health assessment questionnaire.

Table 2. Etanercept levels in good, moderate and non-responders

	Good responders	Moderate responders	Non-responders
1 month	3.40 (2.22–4.62)* †	2.52 (1.26–4.11)†	2.64 (1.20–3.89)*
4 months	3.98 (2.72–5.35)*†	3.08 (2.03–4.52)†	2.54 (1.12–3.94)*
6 months	3.78 (2.53–5.17)*†	3.10 (2.12–4.47)†	2.80 (1.27–3.93)*

Etanercept levels are shown in mg/L
*There was a significant difference between good and European League Against Rheumatism (EULAR) non-responders p=0.009 at 1 month and p<0.001 at 4 and 6 months after start of etanercept treatment.
†There was a significant difference between good and moderate EULAR responders p=0.004 at 1 month, p=0.001 at 4 months and p=0.045 at 6 months after start of etanercept treatment.

In univariate linear regression, an association between etanercept levels and EULAR response was found (regression coefficient 0.54; 95%CI 0.21-0.86, p= 0.001). Confounding analyses were performed by multiple regression analysis. All baseline characteristics were used as potential confounders, no confounders were found.

In addition, we stratified all patients into quartiles according to the height of etanercept level (figure 1). When patients in the highest quartile were compared with patients in the lowest quartile, patients in the lowest quartile were predominantly female (89% vs 68%, p=0.002), used lower doses of methotrexate (12.6±9.9mg/wk vs 16.9±10.1mg/wk, p=0.01), had a higher body mass index (BMI) (27.5±6.3 vs 24.9±4.3, p=0.007) and had a higher glomerular filtration rate (130.0±46.6 vs 107.8±29.4, p=0.001). Logistic regression analyses demonstrated a significant association between the height of serum etanercept levels and EULAR response (OR 2.51; 95%CI 1.58 to 3.98; p<0.001). Correction for the potential confounding variable glomerular filtration rate did not change the results (OR 2.91; 95%CI 1.71 to 4.95; p<0.001).

The percentage of EULAR good responders was significantly different between the highest and the lowest quartiles (p<0.001). The same was true for EULAR non-responders (p=0.001) (figure 1).

Anti-etanercept antibodies were not detected in any of the sera.

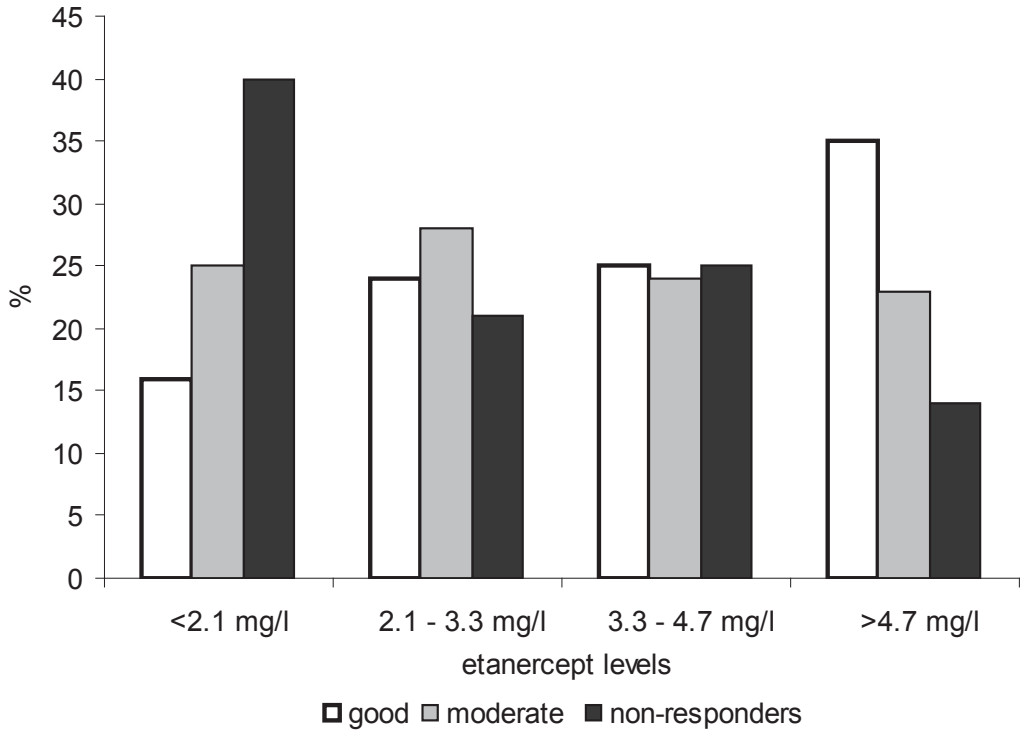

Figure 1. Etanercept levels in good, moderate and EULAR non-responders. This figure represents the percentages of good, moderate and EULAR non- responders according to the height of serum etanercept levels. Each group contains 25% of all patients.

DISCUSSION

This study demonstrates for the first time a clear correlation between height of the serum etanercept level and clinical response. Patients with RA who did not respond to etanercept treatment achieved lower etanercept levels compared with responding patients. We observed that 40% out of all non-responding patients had an etanercept level below 2.1 mg/L, which is considerably lower than the average concentration of 3 mg/L found in pharmacokinetic studies.[16 17] However, as the dose increase in patients with suboptimal response based on clinical data alone was not effective,[1 2] our data suggest that it might be useful to assess the effect of dose increase only in non-responding patients with the lowest etanercept levels (<2.1 mg/L). This personalized treatment approach could be effective and needs to be investigated further. Moreover, we demonstrated that 35% of all responding patients had an etanercept level above 4.7 mg/L; in these patients etanercept dose reduction or interval extension might be possible without the loss of clinical response.

The elimination routes of etanercept are not well documented. An immune response against a drug is a probable cause of accelerated drug clearance.[6] In the current study, we attempted to measure (neutralizing) antibodies against etanercept and did not detect them. An explanation for the absence of anti-etanercept antibodies could be a less immunogenic structure of etanercept as only the fusion part of molecule may contain neoepitope regions to which an immune response can be directed, this is not the functional part of the molecule. Furthermore, drug interference may lead to the underestimation of the anti-etanercept production, as antibody detection is only possible if anti-etanercept production exceeds the concentration of the drug present in the serum.

In this study, patients with low etanercept levels had a significantly higher BMI and were predominantly women. Furthermore, patients with a moderate response had a higher disease activity before the initiation of etanercept compared with good responders. These findings are consistent with other studies reporting that the pharmacokinetics of etanercept can be influenced by demographic and clinical parameters.[17-19] Taken together, the effectiveness of higher etanercept dosage needs to be assessed in patients with high disease activity as well as with high BMI.

The observational cohort design, missing data, but also drop outs may have led to bias. Therefore, last observation carried forward data were used and we performed a sensitivity analysis using only patients who completed 6 months of the study and results did not change. Furthermore, despite the adjustment for known confounders we were not able to exclude confounding by unmeasured factors, for instance x-ray damage, functional disability and baseline TNF concentrations.

In summary, the differences in etanercept levels between responding and non-responding patients detected in this study underline the importance of therapeutic drug monitoring including measurement of the serum drug levels combined with clinical parameter assessment. We suggest that adjusted dosing regimens might be needed in a selected group of patients and it might be more (cost-)effective to adjust etanercept dosages according to serum etanercept concentrations, taken into account high costs of etanercept. More studies are needed to provide evidence for this approach.

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